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EFFECT OF HEAT STRESS AND BENZOIC ACID AS FOLIAR APPLICATION ON EARLINESS AND NUTRIENTS UPTAKE IN COTTON

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ABSTRACT

Effect of foliar applied benzoic acid on growth, earliness and nutrients' uptake in heat stressed cotton (*Gossypium hirsutum* L.) was studied at Department of Agronomy, University of Agriculture, Faisalabad, Pakistan during Kharif 2014. Experiment was laid out in RCBD with split plot arrangement in three replications with net plot size of 3 m × 4.5 m. Treatments included: (a) two levels of heat stress; plots without polythene sheet (control) and plots covered with polythene sheet at square initiation for one week; (b) six benzoic acid levels; viz. control (no spray), control (water spray), benzoic acid spray at 0.25, 0.50, 0.75 and 1.00 mM. The crop was sown on 75 cm apart ridges and plant to plant distance was maintained at 30 cm. All other agronomic practices were kept normal and uniform. The standard procedures were adopted for recording the data on various cotton parameters. The results showed that imposition of heat stress affected cotton phenology and caused all earliness related parameters to occur earlier than control. Heat stress also reduced cotton plants' ability to uptake nutrients (N,P,K Zn and B). However, when crop was applied with benzoic acid, it ameliorated the negative impact of heat stress. Increase in benzoic acid spray concentration increased all above mentioned parameters but upto some extent and then started to decrease. It was concluded that application of 0.75 mM benzoic acid spray improved cotton performance (growth and nutrients' uptake) under both, natural and heat induced environments. Economic analysis also showed that application of 0.75 mM benzoic acid on cotton crop gave maximum net income and benefit cost ratio.

KEYWORDS: ***Gossypium hirsutum*; abiotic stress; growth regulator; NPK, Zn, B, Pakistan.**

INTRODUCTION

Decreased crop productivity in cotton *Gossypium hirsutum* L. is mainly attributed to high temperatures during its growing season (20). Farmers are

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facing the problems of premature shedding of squares, flowers and bolls from their cotton crop due to different stresses mainly heat stress. Conditions responsible for shedding are moisture excess or deficiency, cloudiness, excessive temperature, nutrient imbalances or nutrient deficiency and damages due to insects and diseases (19). Days taken to appearance of first flower, first fruiting branch, and opening of first boll are the main indicators for cotton earliness that are used to select maturing varieties. Cotton earliness, a quantitative trait, is mainly affected by environment. It has been observed that cotton sown earlier or later than its optimum time showed decline in yield (1). Early sowing resulted in better growth than late sowing due to heat stress (3). Earliest sown cotton got favorable environmental conditions resulting into more yield and yield components than late sown cotton (5).

Cotton is a summer crop, therefore high temperature for longer period of time retards its growth and reproduction resulting in low yield. Cotton grows well in hot climate but very high temperature affects its growth and yield badly. Adverse temperatures can affect all stages of cotton. The temperatures higher than optimum, i.e. above 32°C, badly affected the efficiency of crop in different ways, e.g. inhibition of photosynthesis, crop growth rate, decreased metabolism, pollination and fertilization (27).

Plant growth regulators can enhance the physiological efficiency including photosynthetic ability and improve the source-sink relationship .Thus these regulators boost the translocation of photo-assimilates and help in effective flower formation, fruit and seed development and improve crop productivity (28). Benzoic acid (BZA) increased CO2 fixation, intercellular CO2 concentration, stomatal conductance, transpiration rate, net photosynthetic rate and yield in soybean and brown mustard (9). Fariduddin *et al*. (10) also proposed that pigment contents in wheat seedling were increased due to benzoic acid treatment. So, benzoic acid can be utilized against abiotic stress tolerance to that reported for salicylic acid. Imbibition of seed with benzoic acid or salicylic acid are applied as soil drench enhanced tolerance to a variety of stresses (such as heat, chilling and drought) in bean and tomato plants (25).

The daily average temperature best for cotton growth is 27 to 29°C (22).Heat stress may decrease the concentration of nutrients' in plant parts or decrease the total content of nutrients' in the plants, though effects can vary among nutrients and species. It is necessary to find out the plant responses to heat stresses and their underlying physiological mechanisms, as it can provide insights into how plants may be made to become more heat tolerant (30) by using different substances/plant nutrients (16).

Heat stimulus is a significant abiotic issue that hinders the growth, yield and nutrients' uptake in cotton and benzoic acid foliar application might be beneficial for cotton during heat stress by enhancing its growth and nutrients' uptake. So this study was conducted to see the effect of various levels of benzoic acid on growth earliness and nutrients uptake in stressed cotton.

MATERIALS AND METHODS

This study was conducted at Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. The experiment was laid out in randomized complete block design with split plot arrangement having three replications. Cotton was sown on May 27, 2014 using 20 kg seed per hectare. Net plot size was 3m ×4.5m and seeds were placed at 30cm distance on one side of 75cm apart ridges. Fertilizer was applied @ 200:115:95:25:8.75 kg N:P:K:Zn:B per hectare using urea, di-ammonium phosphate, sulphate of potash, zinc sulphate and borax as sources of fertilizer. One fourth of the N and complete doses of the P, K, Zn and B were applied at the time of crop planting; whereas remaining N was applied in three equal splits, 1/4th at 30-35 days after sowing, 1/4th at squaring stage and 1/4th at boll formation stage. The soil samples were taken for analysis before sowing. Physical and chemical properties of soil were estimated by using standard procedures. Data were collected on following parameters.

Number of days from sowing to first floral bud initiation (squaring)

Number of days from sowing to first floral bud initiation (squaring) was recorded from the ten selected plants of each plot when bud became visible with the naked eye having a pin head size of about 3 mm. The data were recorded from the selected plants when 50% selected plants showed squaring.

Number of days from sowing to appearance of first flower

Number of days from sowing to appearance of first flower were noted from the representative plants when 50 percent of selected plants showed flowering with the creamy white or yellowish color of flower.

Number of days from sowing to first boll splition

This character was noted when the lint was seen within the boll with squares (boll locks) around (cracked boll) and took the average of selected plants for accuracy.

Boll maturation period (days)

This period was calculated by deducting the days taken to flowering from the days taken to boll splition from the sowing of crop.

Number of nodes upto first fruiting branch

They were recorded by calculating the number of nodes from the cotyledonary node called the zero node up to first fruiting branch and took the average of selected plants.

Node above white flower (NAWF)

Nodes above white flower were recorded from the selected plants after one week of flowering and continued after one week till NAWF reached 5. Data were noted weekly in days and average of selected plants was taken.

Number of sympodial branches per plant

Number of sympodial branches per plant were recorded by counting the sympodial branches from selected plants of each plot and average was taken.

Number of monopodial branches per plant

Number of monopodial branches per plant was counted from selected plants at the time of second picking and took the average.

Nitrogen in cotton leaf with petiole sample (%)

Nitrogen was determined by Kjeldhal method (6).

Phosphorus in cotton leaf with petiole sample (%)

For phosphorus determination in plant sample dry ashing method was followed as described by Chapman and Pratt (8). The aliquots were analyzed for phosphorus concentration at 410 nm wavelength on Atomic Absorption Spectrophotometer (Model Thermo S-Series).

Potassium in cotton leaf with petiole sample (mg/g)

For potassium determination in plant sample bi-acid (HNO_3-$HClO_4$) method was followed by Gupta (12) and used calibrated flame photometer (Sherwood Flame photometer 410) to make curve.

Zinc in cotton leaf with petiole sample (mg/kg)

For zinc determination in cotton leaf with petiole, wet digestion method using HNO_3-$HClO_4$ was followed as described by Jackson (15). The aliquots were analyzed for zinc concentration on Atomic Absorption Spectrophotometer (Model Thermo S-Series).

Boron in cotton leaf with petiole sample (mg/kg)

For boron determination in plant sample dry ashing method was followed as described by Chapman and Pratt (8). The filtrate was analyzed for boron concentration at 420 nm wavelength on Atomic Absorption Spectrophotometer (Model Thermo S-Series).

Data collected were statistically analyzed using Fisher's analysis of variance technique (29) and the treatments' means were compared by using Tukey's HSD (Honestly significant difference) test at 5% probability.

RESULTS AND DISCUSSION

All phonological events (Table 2), growth parameters (Table 3) and nutrients' uptake (Table 4), in cotton plant were significantly affected by heat stress. Values for all these recorded parameters were higher in plots where cotton was planted under natural environments than where heat was imposed by covering plots with polythene sheet.

As regards benzoic acid spray, effect varied among various parameters with different doses. Maximum number of days from planting to appearance of first floral bud (44.46)and number of days from planting to appearance of first flower(65.52) was recorded foliar application of benzoic acid (F4) @ 0.75 mM which was statistically at par with F_3 (0.50 mM), F_5 (1.00 mM) and F_2 (0.25 mM). Minimum values were noted in F_0 (no spray). Initiation of first square and its development was temperature dependent mostly. Sarwar *et al*. (24) reported that square initiation is affected by high temperature. However, initiation of squaring was used for the selection of early genotypes as it was not affected by heat (11).Benzoic acid improved the exchange of gases that protected plants from damage. Shaheen *et al*. (26) stated that in early planted cotton, temperature was suitable which promoted the cotton flowering and growth while in late sown cotton the temperature was high that reduced the flowering period and promoted early flowering resulting in reduced seed cotton yield. The key factor affecting crop development is temperature.

Maximum number of days from planting to appearance of first boll splition (105.5) was observed in F_3 (0.50 mM benzoic acid spray) and boll maturation period was maximum (40.87) in F_4 (0.75 mM benzoic acid spray) However, these were statistically at par with all other treatments except control (F_0) no spray. Development of first boll and its splition is an important trait for the determination of early maturity in cotton. Hussain *et al*. (14) reported that boll splition is affected by the fundamental atmospheric temperature. As the temperature increased gradually, boll splition boosted with pre-mature boll opening which results in serious decline in seed cotton yield. Solamani *et al*. (28) documented that plant growth regulators spray increased the boll formation period and boll opening resulting in higher boll weight and size in cotton.

Proper boll maturation period confirms proper development of boll with bigger size and weight having superior quality. Bange *et al*. (4) reported that delaying in planting of cotton, reduced the boll maturation period, boll size and boll weight that caused serious shortfall in seed cotton yield with poor fiber quality as temperature increased. Fariduddin *et al.* (10) concluded that improved exchange of gases is a characteristic of benzoic acid treatment enhancing the net photosynthetic rate, intercellular CO_2, WUE, stomatal conductance and transpiration rate in *Brassica juncea*. It also increased the earliness related parameters in soybean plant as we observed in cotton plants flowering, boll maturation and boll splition.

Maximum number of sympodial (2.408) as well as monopodial branches per plant (23.11) were recorded in F_4 (0.75 mM) which was statistically at par with F_5 (1.00 mM) and F_3 (0.50 mM) (Table 3) Minimum values were observed in F_0 (Table 3).Early planting from March to April in different regions of Punjab produced more sympodial branches than May planted cotton which might have to take abiotic factors such as heat stress (7). Application of benzoic acid as a plant growth regulator increased the vegetative branches in cotton.

Table 1. ANOVA for heat stress and benzoic acid spray on earliness and nutrients' uptake in cotton.

SOV	Replica-tion	Heat stress(H)	Error 1	Benzoic acid foliar spray (F)	H × F	Error 2
DF	**2**	**1**	**2**	**5**	**5**	**20**
Days to first square	0.9676	41.9256**	0.0869	35.0258**	1.8253^{NS}	6.9921
Days to first flower	104.104	422.645**	2.770	86.013**	2.783^{NS}	12.706
Days to first boll spilition	57.15	1682.50*	45.98	150.76*	11.73^{NS}	52.04
Boll maturation period (days)	8.809	198.293*	4.127	54.065*	0.553^{NS}	13.804
Number of monopodial branches	0.04075	7.01279**	0.00136	0.63554**	0.02252^{NS}	0.02970
Number of sympodial branches	4.161	191.366**	1.148	27.256**	2.568^{NS}	2.277
Nodes up to first fruiting branch	0.1090	64.6416*	0.7329	10.0088**	0.7612^{NS}	1.2684
Node above white flower	58.58	5041.00**	18.58	398.47**	1.80^{NS}	32.12
Nitrogen concentration in leaf with Petiole (%)	0.03000	2.15111*	0.03864	0.21548**	0.01407^{NS}	0.00978
Phosphorus concentration in leaf with Petiole (%)	0.00074	0.02723**	0.00010	0.02882**	0.00012^{NS}	0.00021
Potassium concentration in leaf with Petiole (mg/g)	0.1531	35.2638**	0.1528	58.1999**	0.1349^{NS}	0.1777
Zinc concentration in leaf with Petiole (mg/kg)	4.658	314.589**	1.134	82.058**	0.150^{NS}	0.995
Boron concentration in leaf with Petiole (mg/kg)	7.032	362.585**	0.300	101.369**	0.450^{NS}	1.202

*****Significant, **Highly significant, NS = Non-Significant, SOV = Source of variation, DF = Degree of freedom

The (Table 3) further elucidates that number of nodes up to first fruiting branch was maximum (11.65)when cotton crop was foliar sprayed with 0.75 mM benzoic acid and it differed non significantly from other foliar applied doses of benzoic acid. Node number upto the first fruiting branch is a morphological character of earliness (18) which determines boll position on stem. Lesser node number up to first fruiting branch will lead to early maturity of cotton and vice-virsa. The node above white flower as an indicator of maturity in cotton. Node number above white flower is consecutively being used to observe development and maturity of cotton crop.

Table 2. Effect of heat stress and benzoic acid spray on some phenological events of cotton

Treatments	DFS	DFF	FBS	BMP
Heat stress (H)				
H_0 (Control)	42.06a	64.02a	105.5a	39.58a
H_1 (Polythene sheet)	39.90b	57.17b	91.9b	34.88b
HSD (≤ 0.05)	0.423	2.390	9.74	2.918
Benzoic acid levels (F)				
F_0 (No spray)	37.80b	55.33c	91.2b	333.05b
F_1 (Water spray)	38.94b	57.59bc	97.1ab	34.92ab
F_2 (0.25 mM)	40.52ab	59.92abc	102.0ab	36.40ab
F_3 (0.50 mM)	42.35ab	63.64ab	105.5a	38.18ab
F_4 (0.75 mM)	44.46a	65.52a	100.3ab	40.87a
F_5 (1.00 mM)	41.84ab	61.57abc	95.9ab	39.96a
HSD (≤ 0.05)	4.799	6.469	13.09	6.743

DFS = Days to first square, DFF = Days to first flower, FBS = Days to first boll splition, BMP = Boll maturation period , Means not sharing a common letter within a column differ significantly at 5% probability level.

Value for nodes above white flower (NAWF) was maximum (101.67) in F5 (1.00 mM benzoic acid) although it differed non-significantly from F3 and F4 (0.75 and 0.50 mM benzoic acid). In both cases control (no spray) remained at bottom followed by water spray. The load of mature bolls increased due to heat stress, it exerted pressure on main stem causing slower development of main stem nodes and white flower appeared at the apex of the plant. Seven or more NAWF should be maintained for four to five weeks for efficient yield. So, first four to five weeks period of flowering is critical to judge physiological cut out stage (NAWF = 5). Decrease in NAWF indicates more movement of assimilates towards boll development than to vegetative growth, so less photosynthesis available at physiological cut out stage of cotton crop. When average node above white flower is 5, this will be last harvestable bloom. The bolls of NAWF = 5 become mature when they accumulate 350 HU (heat units) after anthesis. So NAWF = 5 is called the physiologically cut off stage. Solamani *et al*. (28) claimed that plant growth regulators can enhance the physiological efficiency including photosynthetic ability and improve the source-sink relationship and thus boost the translocation of photo-assimilates and help in effective flower formation, fruit and seed development and improved productivity of the crops.

Table 3. Effect of heat stress and benzoic acid spray on some growth parameters in cotton.

Treatments	NMB	NSB	NUFFB	NAWF
Heat stress (H)				
H_0 (Control)	2.522a	22.74a	11.13a	103.00a
H_1 (Polythene sheet)	1.639b	18.13b	8.45b	79.33b
HSD (≤ 0.05)	0.0530	1.539	1.229	6.192
Benzoic acid levels (F)				
F_0 (No spray)	1.493c	17.89c	7.82c	80.00d
F_1 (Water spray)	1.999b	18.39c	8.98b	84.83cd
F_2 (0.25 mM)	2.060b	19.61bc	9.89ab	88.83bcd
F_3 (0.50 mM)	2.301ab	21.33ab	10.19ab	93.83abc
F_4 (0.75 mM)	2.408a	23.11a	11.65a	97.83ab
F_5 (1.00 mM)	2.224ab	22.28ab	10.19ab	101.67a
HSD (≤ 0.05)	0.3128	2.738	2.044	10.285

NMB = Number of monopodial branches, NSB = Number of sympodial branches, NUFFB = Nodes upto first fruiting branch, NAWF= Node above white flower, Means not sharing a common letter within a column differ significantly at 5% probability level

The results (Table 4) shows that application of benzoic acid increased the concentration of all nutrients' in cotton, over control (no spray) or water spray. Concentrations of P (0.37%), Zn (24.34%) and B (36.24%) were significantly higher with 0.75 mM benzoic acid spray than all other applied doses of benzoic acid. Concentration of N (2.51%) and K (13.11%) (in cotton leaf with petiole) were also highest with 0.75 mM benzoic acid spray however, these were at par with 1.00 mM benzoic acid spray.

The uptake of some macro and micro nutrients, especially nitrogen, potassium, ferrous and boron is limited during heat and drought conditions or by straight forward destruction by the heat stress to roots. According to Akram and Ashraf (2) foliar application of benzoic acid as a plant growth regulator (PGRs) is a well-recognized technique to alleviate abiotic stresses such as heat. Hassanein *et al*. (13) observed an increase in phosphorus and potassium content in wheat plants by the foliar application of benzoic acid plant growth regulator and seed priming under both normal as well as drought and heat stress conditions. Rennenberg *et al*. (23) described that heat stress affects plant nutrient relations. Heat stress may decrease the concentration of nutrients in plants parts or reduce the total content of nutrients especially macro nutrients such as nitrogen, phosphorus and potassium in the plants. Lopez-Bellido *et al*. (17) reported that benzoic acid and other plant growth regulators such as paclobutrazol and trinexapac-ethyl increased the organic soil carbon and increased the cation exchange capacity of soil and thus increasing the ability to capture and uptake the essential plant micro and macro nutrients such as Ca, Mg and K.

Table 4. Effect of heat stress and benzoic acid spray on nutrients' uptake in cotton.

Treatments	NCLP (%)	PCLP (%)	KCLP (mg/g)	ZnCLP (mg/kg)	BCLP (mg/kg)
Heat stress (H)					
H_0 (Control)	2.47a	0.31a	10.63a	22.23a	34.17a
H_1 (Polythene sheet)	1.98b	0.25b	8.65b	16.32b	27.83b
HSD (≤ 0.05)	0.282	0.014	0.562	1.529	0.787
Benzoic acid levels (F)					
F_0 (No spray)	1.99d	0.19e	5.10e	14.42e	25.01d
F_1 (Water spray)	2.07d	0.22d	7.17d	16.25d	28.07c
F_2 (0.25 mM)	2.16cd	0.26c	9.04c	18.39c	30.01c
F_3 (0.50 mM)	2.26bc	0.31b	11.08b	20.05c	32.78b
F_4 (0.75 mM)	2.51a	0.37a	13.11a	24.34a	36.24a
F_5 (1.00 mM)	2.36ab	0.33b	12.35a	22.20b	33.89b
HSD (≤ 0.05)	0.179	0.026	0.765	1.810	1.989

NCLP= Nitrogen concentration in leaf with petiole, PCLP= Phosphorus concentration in leaf with petiole, KCLP= Potassium concentration in leaf with petiole, ZnCLP= Zinc concentration in leaf with petiole, BCLP= Boron concentration in leaf with petiole

Table 5. Economic analysis of various treatments' combinations (H × F).

Treatments	H_1F_0	H_1F_1	H_1F_2	H_1F_3	H_1F_4	H_1F_5
Net Income (Rs. ha^{-1})	20500	30342	59257	73414	88222	84254
BCR	1.21	1.30	1.56	1.68	1.80	1.75
Treatments	H_0F_0	H_0F_1	H_0F_2	H_0F_3	H_0F_4	H_0F_5
Net Income (Rs./ha)	77212	75944	87941	98594	141306	120952
BCR	1.68	1.72	1.82	1.90	2.23	2.05

Means not sharing a common letter within a column differ significantly at 5% probability level.
H_0 = No heat stress H_1 = Heat stress, F_0 = No spray, F_1 = Water spray, F_2 = 0.25 mM benzoic acid spray, F_3 = 0.50 mM benzoic acid spray, F_4 = 0.75 mM benzoic acid spray
F_5 = 1.00 mM benzoic acid spray

Pakistani soils are normally alkaline in nature and show deficiencies of micronutrients especially of zinc (Zn), iron (Fe) and boron (B) in different portions of the country and a decrease in zinc content due to heat stress and dilution effects. Akram and Ashraf (2) claimed that PGRs including benzoic acid improved the concentration of zinc in the petiole that increased the yield. Zinc and boron shortage in many horticultural and agronomic crops is due to heat stress and alkaline soils (21) that can be improved through foliar application of benzoic acid as a plant growth regulator (13).

To look into the feasibility of benzoic acid foliar application on cotton crop, economic analysis was carried out keeping in view the current market prices. The data (Table 5) showed that maximum net income and benefit cost ratio can be obtained by foliar application of 0.75 mM benzoic acid on cotton crop.

Pic. 1. Effect of heat stress on crop growth and fiber quality of cotton

CONCLUSION

Growth and nutrients' uptake in cotton were negatively affected by heat stress. Foliar applied benzoic acid helped ameliorate these negative impacts Banzoic acid @ 0.75 mM benzoic acid proved best in this regard. Maximum net income and BCR were also obtained when crop was sprayed with 0.75 mM BZA solution under both heat and no heat induced conditions.

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Muhammad Farrukh Saleem	**:**	**Planned experiment**
Shakeel Ahmad Anjum	**:**	**Provided experimental material**
Muhammad Ashfaq Wahid	**:**	**Provided technical assistance**
Muhammad Tariq Saeed	**:**	**Analysed data and prepared write-up**